THE IMPACT OF AGE ON QUANTITATIVE INFRARED PUPILLOMETRY IN HEALTHY CHILDREN 1-18 YEARS

BY

JACOB TYLER BROWN
PharmD, Creighton University School of Pharmacy, 2010

Submitted to the graduate degree program in Clinical Research and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science

_____

Chairperson Theresa I. Shireman, RPh, PhD

_____

Susan Abdel-Rahman, PharmD

_____

Sue-Min Lai, PhD

Date Defended: April 8, 2014

The Thesis Committee for JACOB TYLER BROWN, PharmD
certifies that this is the approved version of the following thesis:

THE IMPACT OF AGE ON QUANTITATIVE INFRARED PUPILLOMETRY IN HEALTHY CHILDREN 1-18 YEARS

_____

Chairperson Theresa I. Shireman, RPh, PhD

Date approved: April 8, 2014

ABSTRACT

OBJECTIVE: The goal of this study was to establish normative values under ambient light conditions for measurements of quantitative pupillometry in children.

METHODS: This was a cross-sectional analysis of pupillometry values obtained in children. Quantitative pupillomentry measurements were obtained from children between 1 and 18 years of age being seen for either a well child check or other outpatient appointment. Participants were excluded if they were in pain, on any medication known to affect pupil size (i.e. opioids, stimulants), or had any chronic neurologic conditions.

RESULTS: A total of 242 children were enrolled in this study, with pupillometry readings obtained from a total of 171 children after exclusions. Maximum and minimum pupil size increased slightly with age; however, the correlation was weak (r = 0.14 and r = 0.16). Similarly weak correlations with age also were observed for maximum constriction velocity (r = -0.12) and dilation velocity (r = 0.05). No differences were observed between males and females for any of the pupil parameters. Maximum (5.35 mm vs. 4.91 mm) and minimum (3.71 mm vs. 3.36 mm) pupil sizes were significantly larger in Whites than African-American participants.

CONCLUSIONS: Pupil size and reactivity show little correlation with age, appear to be relatively independent of ontogeny, and therefore would not be expected to significantly impact further exploration in utilizing pupillometry as a biomarker across the pediatric age range. Differences in race should be taken into consideration when pupillometry is used in mixed populations.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my academic advisor and thesis committee chair, Dr. Theresa Shireman, for her continual guidance over the past two years. I would also like to thank the other thesis committee members, Dr. Susan Abdel-Rahman, and Dr. Sue-Min Lai for all the assistance and direction they have provided to me with this research project.

Additionally, I would like to acknowledge Drs. Kathleen Neville and Mark Connelly for guiding me throughout the design, enrollment, and analysis of this research. Additionally, I would like to thank Jaylene Weigel and Caitlin Nichols for their help with participant enrollment.

TABLE OF CONTENTS

Introduction

Pupillometry is defined as "the measurement of variations in the diameter and the pupillary aperture of the eye," and includes the following parameters: maximum (resting) and minimum (following light stimulus) pupil diameter, average and maximum constriction velocity, and dilation velocity (following light stimulus).[1] The clinical usage of pupillometry has gained renewed interest as a clinical biomarker, largely due to the introduction of user-friendly, reliable, and portable measurement devices.[2] This development of infrared pupillometers has removed much of the subjectivity involved in quantifying pupil dynamics, making readings of pupil size and reactivity simple, efficient, and extremely accurate. These advances have allowed for novel applications of pupillometry by clinicians in pharmacology and critical care research.

In response to these advancements, pupillometry has been applied in several experimental settings, including monitoring for early changes in intracranial pressure (via the pupillomotor nuclei in the dorsal midbrain and the oculomotor nerve),[4] associating oxycodone and fentanyl plasma concentrations with pupil size (opioids constrict the pupil via cortical inhibition of the Edinger-Westphal nucleus),[5-7] and correlating subjective pain scores with a standardized pupillary dilatation reflex (in response to a noxious stimuli) in post-operative patients from alterations in sympathetic and parasympathetic tone.[8] Additionally, pupillometry has been shown to correlate with the efficacy of tramadol in healthy volunteers, serving as a surrogate for CYP2D6 activity and potentially identifying individuals who may not have the intended therapeutic effect with standard dosing of the drug,[9] with similar findings shown with alfentanil and CYP3A4 activity.[10]

In order to accurately assess pupil size and reactivity values obtained in children, it is necessary to consider how pupil parameters obtained under experimental conditions compare to pupil parameters in healthy children. Several prior studies have evaluated normal pupil size and reactivity in children in a variety of ways.[11-14] MachLachlan and Howland describe normal values under low light conditions for pupil diameters in children down to one month of age; however, subjects were limited to only pupil

diameter due to the photographic technology utilized.[11] Taylor et al. reported pupil size and reactivity values under ambient light conditions in healthy volunteers from 1-87 years of age, but reported group means rather than examining changes with age.[12] Kohnen et al. reported low light pupil size and reactivity utilizing a Colvard infrared pupillometer but were limited in total sample size (n=83) and measurements in older children.[13] Finally, Boev et al. describe normative pupillometry data in healthy pediatric volunteers (n=90) from 1-18 years of age under ambient light conditions utilizing an older Neuroptics pupillometer (Forsite); however, they reported their data in age groups (0-2, 2-6, 6-12, 12-18 years), making it difficult to assess changes with age in a detailed manner.[14]

The purpose of this study was to further characterize normal pupil size and reactivity under ambient light conditions, assess the feasibility of using pupillometers in children down to 12 months of age (i.e. how realistic it is to obtain readings from a pupillometer across an age spectrum), and determine differences (if any) in age, sex, and race.

Methods

Study design

This was a cross-sectional study of healthy male and female children between the ages of 1 and 18 years using convenience sampling at three area pediatric clinics. This study was approved by the Institutional Review Board with oversight for all locations. Participants were enrolled with informed permission/assent.

Sample population

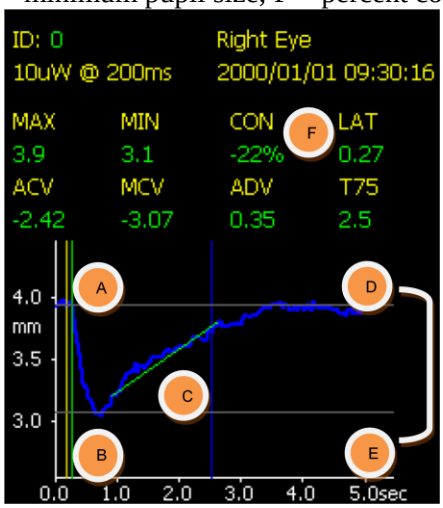
Participants were recruited during well child checks (regularly scheduled outpatient visits for assessment of growth and development) or during other outpatient clinic appointments. Participants were excluded if they had any neurologic illness that may affect pupil size, were presently in pain defined as a pain score greater than one (as expressed by either the participant or parent/guardian), were taking any

medication known to affect pupil size (opioids, stimulants, or anticholinergics), or were unwilling/unable to participate.

Outcome measures

Pupil size and reactivity data was obtained using a Neuroptics PLR-200 pupillometer (Neuroptics, Inc, Irvine, California) under ambient light conditions. This device is a handheld, digital infrared, monocular pupillometer that auto-focuses, auto-calibrates, and controls for vertex distance. It measures the best circular fit of the pupil, records 30 measurements per second, and reports mean values while excluding outliers. As shown in a recent study, the Neuroptics pupillometer has a high interobserver agreement and repeatability.[3] For the reading, a rubber cup on the pupillometer is placed around the child's eye to block out peripheral light. Once the pupil is detected, the pupillometer determines the resting (maximum) pupil diameter (mm), flashes a brief standardized light stimulus, and then determines the resulting average and maximum pupil constriction velocity (mm/s), minimum pupil diameter (mm), time to minimum diameter (s), percent constriction (resting minus minimum diameter), and dilation velocity (mm/s) (Figure 1, used with permission).

Figure 1. Neuroptics PLR-200 Pupillometer output
A and B = maximum and average constriction velocity; C = dilation velocity; D = maximum pupil size; E = minimum pupil size; F = percent constriction



The validity of each reading is displayed in the output and determined by the software integrated within the device. A maximum of three attempts were made in each individual. If more than one reading was obtained, the average of those readings is reported. If no valid readings were obtained after three attempts, no additional readings were attempted.

Covariates

Parents or guardians completed a self-report questionnaire in order to ascertain the participant's age, race, ethnicity, current state of pain (based on a scale from 1-10), any known illness, and current medications.

Analysis plan

The data were analyzed by descriptive statistical methods. Maximum and minimum pupil sizes are presented as means with standard deviation categorized as yearly age groups. One way ANOVA tests were used to compare pupil parameters of all races, while independent t-tests were used for direct comparisons when comparing pupil parameters between sex and race (White vs. African-American, White vs. Other, and African-American vs. Other). Chi-square tests were used to compare differences between valid and invalid readings. Pearson's correlation test (r) was utilized in order to assess the linear relationship between age and pupil parameters.

Results

A total of 242 participants were approached for study participation. Of these, 7 participants were ineligible due to being on a medication known to affect pupil size and reactivity, while 7 reported pain scores of greater than 1 (based on a scale of 1-10). Fifty-seven participants were excluded due to invalid readings as determined by the software. No participants were excluded due to neurologic illness. Nearly half (47%) of the readings deemed as invalid by the software occurred in children under 5 years of age (p

< 0.01), while gender and race in participants with excluded readings was nearly identical to the overall population ($p > 0.05$) (Table 1).

Table 1. Demographics of valid vs. invalid readings

Demographics	Valid	Invalid	Percent Invalid	P-value
Age ≤5y	34	27	44	0.0001
Age >5y	137	30	18	
Gender*				
Male	85	27	24	0.9681
Female	86	29	25	
Race				
White	100	38	28	0.5406
Black	47	13	22	
Other	24	6	20	

Data from 171 participants (50% male) were analyzed, with a self-identified race distribution of the enrolled sample of 59% White, 28% African-American, and 13% other. Of the total population, 19% self-identified as Hispanic.

Average maximum and minimum pupil sizes by age are displayed in Table 2. Overall, both maximum and minimum pupil size were shown to increase slightly for the entire population until around 11 years of age and subsequently plateau; however, this finding was not found to be statistically significant for maximum pupil size and the linear correlation between age and maximum/minimum pupil size was very weak ($r = 0.14$, slope (b) = 0.81, 95% confidence intervals (CI) = -0.04-1.66; $p = 0.06$, and $r = 0.16$, $b = 1.20$, 95% CI = 0.08-2.31; $p = 0.04$). Weak and non-significant correlations with age were also observed for maximum constriction velocity ($r = -0.12$, $b = -0.67$, 95% CI = -1.49-0.15; $p = 0.11$) and average dilation velocity (usable $n = 140$) ($r = 0.05$, $b = 0.90$, 95% CI = -1.89-3.68; $p = 0.53$) (Figure 2). Average constriction velocity also showed a weak correlation with age ($r = -0.22$, $b = -1.60$, 95% CI = -2.67- (-)0.53; $p < 0.01$), as did percent constriction ($r = 0.10$, $b = 0.09$, 95% CI = -0.05-0.23; $p = 0.21$).

Figure 2. Scatter plot of maximum constriction velocity and dilation velocity

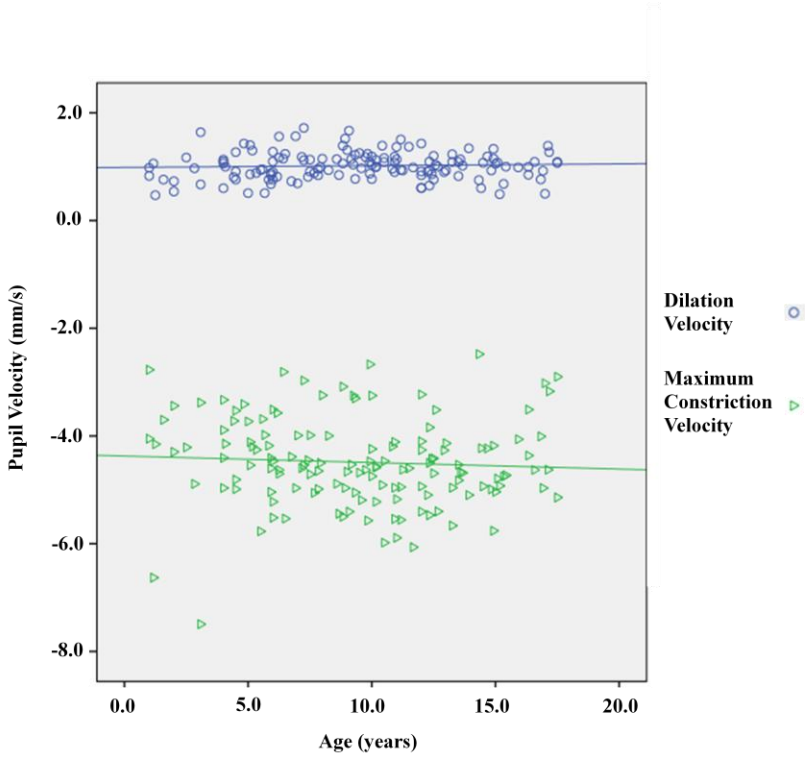


Table 2. Average values of maximum and minimum pupil sizes

Age (years)	Number of participants	Maximum Pupil Size (mm (SD))	Minimum Pupil Size (mm (SD))
1-2	8	4.82 (1.13)	3.44 (0.71)
2-3	7	4.64 (0.84)	3.10 (0.64)
3-4	6	5.02 (0.83)	3.28 (0.73)
4-5	13	5.27 (0.60)	3.50 (1.09)
5-6	16	4.90 (0.60)	3.34 (0.53)
6-7	14	5.11 (0.73)	3.52 (0.61)
7-8	11	5.31 (0.87)	3.73 (0.65)
8-9	8	4.99 (1.02)	3.42 (0.66)
9-10	12	5.16 (0.92)	3.62 (0.74)
10-11	17	5.55 (0.46)	3.83 (0.42)
11-12	10	5.66 (0.30)	3.70 (0.37)
12-13	16	5.19 (0.72)	3.59 (0.49)
13-14	9	5.59 (0.63)	3.90 (0.55)
14-15	7	5.41 (1.04)	3.90 (0.65)

15-16	7	5.34 (0.41)	3.67 (0.50)
16-17	5	5.07 (0.48)	3.61 (0.51)
17-18	5	4.37 (1.12)	3.18 (0.65)
Total / Average	171	5.19 (0.77)	3.57 (0.64)

No differences were observed between males and females for any of the pupil parameters. One way ANOVA tests comparing race and each pupil parameter revealed significant differences for maximum ($p = 0.02$) and minimum ($p < 0.01$) pupil size. Mean maximum pupil size was significantly larger in Whites than African-American participants (5.35 mm vs. 4.91 mm) ($p < 0.01$), as was minimum pupil size (3.71 mm vs. 3.36 mm) ($p < 0.01$) (Table 3).

Table 3. Mean comparisons of gender and race

Mean Pupil Parameter	Male	Female	White	Black
Maximum Pupil Size (mm)	5.18 (0.78)	5.19 (0.76)	*5.35 (0.69)	*4.91 (0.87)
Minimum Pupil Size (mm)	3.58 (0.55)	3.59 (0.62)	*3.71 (0.55)	*3.36 (0.58)
Percent Change (%)	-30.49 (4.98)	-30.55 (4.78)	-30.34 (5.04)	-30.98 (4.77)
Average Constriction Velocity (mm/s)	-3.38 (0.63)	-3.44 (0.57)	-3.46 (0.61)	-3.35 (0.60)
Maximum Constriction Velocity (mm/s)	-4.45 (0.87)	-4.53 (0.74)	-4.55 (0.79)	-4.39 (0.87)
Dilation Velocity (mm/s)	1.01 (0.22)	1.02 (0.29)	1.01 (0.24)	1.07 (0.24)

*p-value < 0.05

When stratified by race, correlations were statistically significant for maximum pupil size and age in Whites ($r = 0.26$, $b = 1.60$, 95% CI = 0.40-2.80; $p = 0.01$) but not African-Americans ($r = 0.10$, $b = 0.53$, 95% CI = -1.11-5.16; $p = 0.52$) (Figure 3). Similarly, minimum pupil size and age was statistically significantly (though weakly correlated) in Whites ($r = 0.26$, $b = 2.02$, 95% CI = 0.53-3.51; $p = 0.01$) but not African-Americans ($r = 0.13$, $b = 1.09$, 95% CI = -1.34-3.51; $p = 0.37$) (Figure 4). No differences in any of the pupil parameters were observed when comparing the other racial groups.

Figure 3. Maximum pupil sizes for Whites and African-Americans.

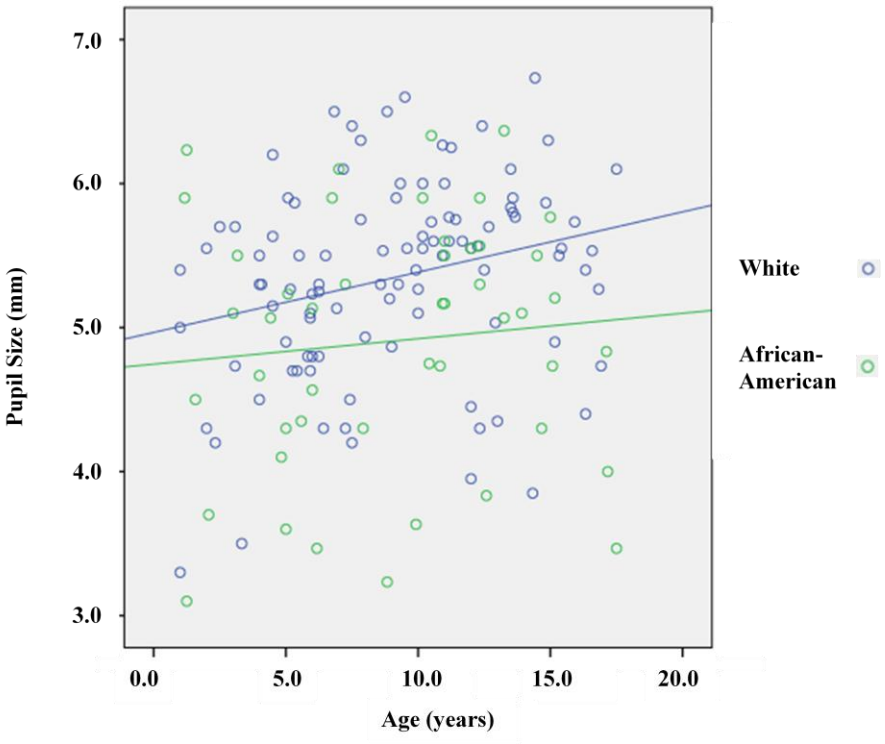
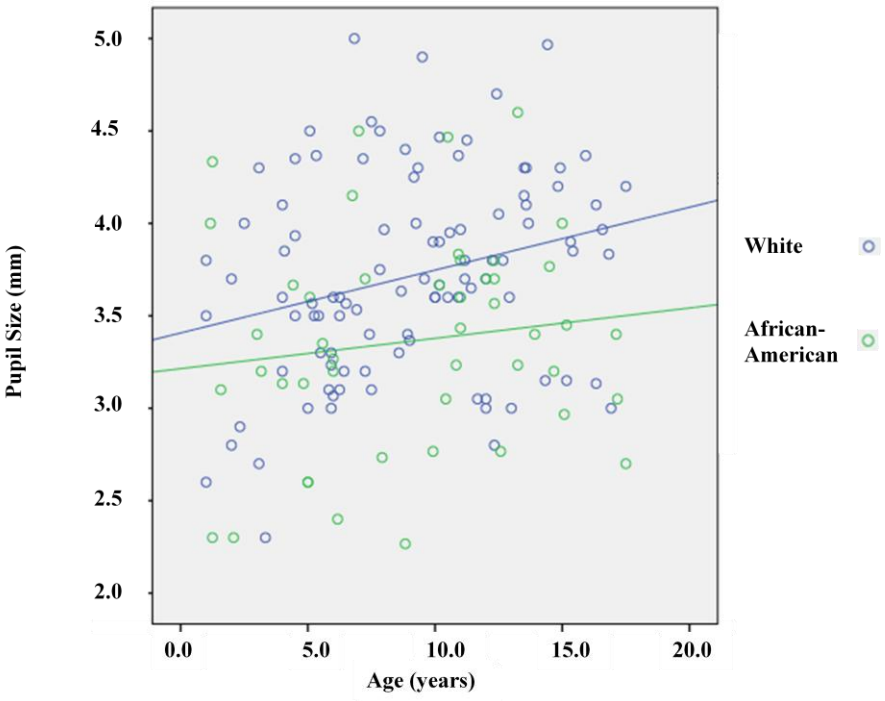


Figure 4. Minimum pupil sizes for Whites and African-Americans.



Discussion

Advances in the technology of pupillometers have led to an increased utilization of pupillometry as an assessment tool in several areas. Establishing normative values in pediatrics is imperative to accurately assist in the interpretation of pupil response in pediatric studies that employ pupillometry as a tool. This study is the first to provide quantitative data of pupil size and reactivity under ambient light conditions across a representative sample of healthy children of all ages. These results add to the existing body of literature on normal ranges of pupil size and reactivity throughout child development, showing a slight increase in maximum and minimum pupil size with age. Dilation velocity showed little change over the pediatric age range, while maximum constriction velocity portrayed a slight decrease. Additionally, significant differences were found in two parameters (maximum/minimum pupil) when comparing White and African-American children (Table 3), suggesting that race should be considering when interpreting pupillometry data. Interestingly, Whites also had higher correlations between maximum/minimum pupil size and age as compared to African-Americans, although their magnitude was weak.

It also is important to note that no differences between males and females were found in pupil parameters, consistent with a prior report in adult participants 18-80 years of age using the same brand of pupillometer indicating that no gender differences were found in pupils adjusted to dim light settings.[15] Several variables were compared in order to identify subpopulations in whom this device may be more or less feasible. Of the three demographics (age, gender, and race) compared between valid and invalid readings, gender and race showed comparable proportions between the two groups. In contrast, a significantly higher proportion of invalid readings occurred in children less than 5 years of age, suggesting that the feasibility of obtaining valid readings is impacted by age, and that younger age is an important consideration when anticipating obtaining pupillometry readings. Additionally, although data regarding iris color was not included in this study, a recent study showed no variation between individuals with different iris colors.[15]

While newer pupillometers do allow for simple attainment of measurements, the reliability of the device is an important consideration and has been addressed by several studies. Meeker *et al.* compared measurements taken manually by several different clinicians (neurosurgeons, neurosurgical interns, and advanced practice nurses) to those obtained with an automated, portable pupillometer. They demonstrated that pupil measurements obtained via an infrared pupillometer have much lower inter-examiner disagreement and were also able to detect pupillary changes earlier than with manual examination.[16] When comparing three different brands of pupillometers, the Neuroptics pupillometer demonstrated better reliability and agreement between users as compared to the other two.[3] An additional study comparing the Neuroptics PLR-200 with the laboratory standard of infrared photography showed clinically satisfactory accuracy, with none of the readings showing a difference greater than +/- 0.5 mm. [17]

As several neuroanatomical pathways contribute to pupil size and reactivity, numerous clinical applications may be possible. Pupillary response to pain and analgesia has been proposed as an objective tool for pain assessment and has been shown to be a more sensitive marker of pain and analgesic drug response in children and adults when compared to traditional hemodynamic markers.[18,19] Capturing the extent of pupil dilation can provide an index of acute nociceptive input via autonomic innervation of the iris muscles, [20] while capturing the extent of reduction in this pupillary response during exposure to opioid pain medicine can provide an index of a given drug's pharmacological effect by reflecting the extent of occupancy of mu and kappa opioid receptors in the central nervous system.[18] Furthermore, pain assessment in children or non-verbal patients is often difficult to accurately assess, and pupillometers may offer a new tool for advancing the understanding of individual differences in pain and analgesic response in children by providing an objective measurement of pain and analgesia.

While approximate values for age ranges can be estimated from the data, we found a considerable amount of inter-individual variability for maximum and minimum pupil sizes. As a result, it may be likely that the most relevant information is gained by examining changes in an individual's own dynamic values

(i.e., constriction/dilation velocity, percent constriction) or change in pupil size from their baseline values. Furthermore, this study shows that pupil readings are possible in younger children down to 12 months of age, although as previously stated valid measurements were more difficult to obtain for children less than 5 years of age. In order to increase the likelihood of obtaining a reading from a child less than 5 years, parents assisted in helping to steady the child's head long enough to obtain a reading. Although an advantage of the pupillometer is its ease of use, patient-operator interaction and operator proficiency is still required when obtaining readings. The development of a pupillometer requiring less active participation for obtaining readings in younger children (e.g., obtaining readings from a distance, software that integrates motion better, etc.) may be useful to capture data in younger children.

This study adds important information to the existing knowledge related to normal pupil size and reactivity in healthy children. Due to methodological differences, previous studies in this area have produced varying results. MachLachlan et al. used a large sample size of participants 1-19 years of age to quantify typical pupil parameters; however, their results were limited to only resting pupil diameter and interpupillary distance under low light conditions.[11] Similarly, Kohnen et al. reported only mean pupil size values under scotopic conditions in children between 0-15 years of age; of note, these researchers similarly found an increase in pupil size until approximately 11 years of age.[13] Taylor et al. reported size and reactivity data in healthy volunteers between 1-87 years of age but collapsed data across the entire age range.[12] Lastly, Boev et al. reported normative quantitative pupillometry data in 90 children 1-18 years of age.[14] Results from the Boev et al. study contrasted with our findings in that mean values for maximum and minimum pupil size from our study were consistently larger.

The study was limited by the exclusion of children less than one year of age as well as the convenience sampling utilized for enrollment, which may introduce an element of systematic bias. Given that a significant amount of neurodevelopment occurs prior to one year of age, it is possible that pupil size and/or reactivity may appreciably change throughout this timeframe. Our participant population had a disproportionate number of White participants as compared to African-Americans. Additionally, because

ambient light was not explicitly defined in this study some of the variability may be due to differences in ambient lighting between the different enrollment locations.

It is important that future studies in pupillometry take into consideration as many factors as possible that could affect pupil size, including patient population demographics as well as lighting in the environment readings are taken. Future studies to assess quantitative puillometry in children less than one year of age will likely require technological advancements in the way these readings are obtained. Furthermore, studies examining pupil size and reactivity in neurological disorders and medications that affect pupil dynamics are also needed.

In conclusion, pupil size and reactivity as quantified by infrared pupillometry appear to be relatively independent of ontogeny in children greater than one year of age, and therefore age would not be expected to significantly impact future studies utilizing pupillometry as a biomarker across the pediatric age range. Due to the absence of significant changes with age, extrapolation of adult pupillometry data to the pediatric population may be reasonable. Additionally, future studies utilizing pupillometry in mixed racial populations must take into account that differences may exist. These data provide an important baseline and are critical considerations for interpretation of future pediatric studies using pupillometry as a pharmacodynamic biomarker.

References

1. Andreassi JL. (2007). Psychophysiology: Human Behavior and Physiological Response. Mahwah, New Jersey: Lawrence Erlbaum Associates, Inc., Publishers; p 350-351.

2. Fountas KN, Kapsalaki EZ, Machinis TG, Boev AN, Robinson JS, Troup EC. (2006). Clinical implications of quantitative infrared pupillometry in neurosurgical patients. *Neurocritical care,* 5(1), 55-60.

3. Schallenberg M, Bangre V, Steuhl KP, Kremmer S, Selbach JM. (2010). Comparison of the Colvard, Procyon, and Neuroptics pupillometers for measuring pupil diameter under low ambient illumination. *Journal of refractive surgery,* 26(2), 134-143.

4. Chen JW, Gombart ZJ, Rogers S, Gardiner SK, Cecil S, Bullock RM. (2011). Pupillary reactivity as an early indicator of increased intracranial pressure: The introduction of the Neurological Pupil index. *Surgical neurology international*, 2, 82.

5. Benziger DP, Miotto J, Grandy RP, Thomas GB, Swanton RE, Fitzmartin RD. (1997). A pharmacokinetic/pharmacodynamic study of controlled-release oxycodone. *Journal of pain and symptom management*, 13(2), 75-82.

6. Ibrahim AE, Feldman J, Karim A, Kharasch ED. (2003). Simultaneous assessment of drug interactions with low- and high-extraction opioids: application to parecoxib effects on the pharmacokinetics and pharmacodynamics of fentanyl and alfentanil. *Anesthesiology*, 2003, 98(4), 853-61.

7. Macleod DB, Habib AS, Ikeda K, Spyker DA, Cassella JV, Ho KY, Gan TJ. (2012). Inhaled fentanyl aerosol in healthy volunteers: pharmacokinetics and pharmacodynamics. *Anesthesia and analgesia*, 115(5), 1071-7.

8. Aissou M, Snauwaert A, Dupuis C, Atchabahian A, Aubrun F, Beaussier M. (2012). Objective assessment of the immediate postoperative analgesia using pupillary reflex measurement: a prospective and observational study. *Anesthesiology*, 116(5), 1006-12.

9. Matouskova O, Slanar O, Chytil L, Perlik F. (2011). Pupillometry in healthy volunteers as a biomarker of tramadol efficacy. *Journal of clinical pharmacy and therapeutics*, 36(4), 513-7.

10. Phimmasone S, Kharasch ED. (2001). A pilot evaluation of alfentanil-induced miosis as a noninvasive probe for hepatic cytochrome P450 3A4 (CYP3A4) activity in humans. *Clinical pharmacology and therapeutics*, 70(6), 505-17.

11. MacLachlan C, Howland HC. (2002). Normal values and standard deviations for pupil diameter and interpupillary distance in subjects aged 1 month to 19 years. *Ophthalmic and physiological optics*, 22(3), 175-82.

12. Taylor WR, Chen JW, Meltzer H, Gennarelli TA, Kelbch C, Knowlton S, Richardson J, Lutch MJ, Farin A, Hults KN, Marshall LF. (2003). Quantitative pupillometry, a new technology: normative data and preliminary observations in patients with acute head injury. Technical note. *Journal of neurosurgery*, 98(1), 205-13.

13. Kohnen EM, Zubcov AA, Kohnen T. (2004). Scotopic pupil size in a normal pediatric population using infrared pupillometry. *Graefe's archive for clinical and experimental ophthalmology*, 242(1), 18-23.

14. Boev AN, Fountas KN, Karampelas I, Boev C, Machinis TG, Feltes C, Okosun I, Dimopoulos V, Troup C. (2005). Quantitative pupillometry: normative data in healthy pediatric volunteers. *Journal of neurosurgery*, 103(6 Suppl), 496-500.

15. Bradley JC, Bentley KC, Mughal AI, Bodhireddy H, Young RS, Brown SM. (2010). The effect of gender and iris color on the dark-adapted pupil diameter. *Journal of ocular pharmacology and therapeutics*, 26(4), 335-40.

16. Meeker M, Du R, Bacchetti P, Privitera CM, Larson MD, Holland MC, Manley G. (2005). Pupil examination: validity and clinical utility of an automated pupillometer. *The Journal of neuroscience nursing*, 37(1), 34-40.

17. Bradley JC, Bentley KC, Mughal AI, Brown SM. (2010). Clinical performance of a handheld digital infrared monocular pupillometer for measurement of the dark-adapted pupil diameter. *Journal of cataract and refractive surgery*, 36(2), 277-81.

18. Barvais L, Engelman E, Eba JM, Coussaert E, Cantraine F, Kenny GN. (2003). Effect site concentrations of remifentanil and pupil response to noxious stimulation. *British journal of anaesthesia*, 91(3), 347-52.

19. Constant I, Nghe MC, Boudet L, Berniere J, Schrayer S, Seeman R, Murat I. (2006). *British journal of anaesthesia*, 96(5), 614-9.

20. Höfle M, Kenntner-Mabiala R, Pauli P, Alpers GW. (2008). You can see pain in the eye: pupillometery as an index of pain intensity under different luminance conditions. *Internal journal of psychophysiology*, 70(3), 171-5.